the liver-stage of infection. Thus these epitopes will be of value for inclusion in vaccines designed to provide immunity to *Plasmodium falciparum*. Furthermore, this work identifies for the first time CTL epitopes in the *P. falciparum* antigen, thrombospondin-related anonymous protein (TRAP; Robson et al., 1988), and thus identifies TRAP, and/or peptides from TRAP, as a useful component of a CTL-inducing vaccine against *P. falciparum* malaria.

Page 2, line 8, please replace with the following:

SUMMARY OF THE INVENTION

Page 3, line 10, please insert the following:

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT(S)

Page 3, line 13, please replace the paragraph as follows:

We have recently described a novel approach to identifying CTL epitopes and potential CTL epitopes in *P. falciparum* (Hill et al., 1992). This consisted, in brief, of 1) determining a motif for peptides bound to a particular HLA class I molecules, 2) synthesizing peptides from *P. falciparum* antigens congruent with this motif, 3) testing whether these peptides bind to that HLA class I allele using a binding assay known as a HLA assembly assay, and 4) testing whether lymphocytes from individuals exposed to malaria could recognise these peptides as epitopes after suitable in vitro restimulation and culture. In that work we identified peptide epitopes and potential peptide epitopes for two HLA class I molecules, HLA-B53 and HLA-B35. Here we extend that work to four further HLA class I molecules: HLA-A2, HLA-B8, HLA-B7 and HLA-B17. 52 peptides are identified that are shown either to be epitopes or potential epitopes for these HLA class I molecules.

Page 3, line 33 to page 4, line 26, please rewrite the paragraph as follows:

Motifs for eluted peptides.

The peptide binding motifs of HLA-A2, HLA-B7 and HLA-B8 have been described. For HLA-A2 strong preferences were found at positions 2 and 9 of bound peptides: for leucine, isoleucine and methionine at position 2, and for valine, leucine and isoleucine at position 9 (Falk et al., 1991 and Hunt et al., 1992). For HLA-B7 the preferred residues were proline at position 2 and a hydrophobic residue at position 9. Leucine, isoleucine, valine, alanine, phenylalanine and tryptophan were the hydrophobic residues preferred (Huczko et al., 1993). For HLA-B8 preferred residues were found at positions 3, 5 and 9 (Sutton et al., 1993). At positions 3 and 5 lysine or arginine are preferred and at position 9, leucine or isoleucine or valine. The motif for peptides bound to HLA-B17 has not been reported so we determined this exactly as described for HLA-B35 (Hill et al., 1992), using instead of the cell line CIR-B35, the cell line CIR-B58. Hence the peptide motif determined is strictly for HLA-B58 which along with HLA-B57 constitutes a subtype of HLA-B17. The primary amino acid sequences of HLA-B57 and HLA-B58 are very similar indicating that the types of peptides bound are likely to be very similar, at least in their key anchor residues. Hence we refer to the motif determined for CIR-B58 as a motif for HLA-B17. For HLA-B17, preferred amino acids were observed at positions 2 and 9: serine and threonine were preferred at positions 2 and a hydrophobic residue (defined as for B7 above) at position 9.

Page 4, line 29 to page 5, line 5, please rewrite the paragraph as follows:

As described previously for identification of HLA-B35 and HLA-B53 epitopes (Hill et al., 1992), peptides were synthesised to correspond to these four motifs from the primary amino acid sequences of four *P. falciparum* pre-erythrocytic antigens: CSP, TRAP, LSA-1 and SHEBA. Over one hundred peptides were synthesized. Binding assays (Elvin et al., 1992) were performed on selected peptides to determine whether they bound to HLA-A2, -B7, -B8, or -B58 using the untransfected T2 cell line (for HLA-A2) or the T2 cell line transfected with HLA-B7 (used to assess binding to HLA-B7s), or with HLA-B8 (used to assess binding to HLA-B8), or with HLA-B58 (used to assess binding to HLA-B58).

Page 5, line 8, please rewrite the paragraph as follows:

Peptides shown to bind to a particular HLA class I allele were tested for CTL recognition in assays using lymphocytes from malaria-exposed Gambians, as described previously (Hill et al., 1992). A minority of peptides, synthesized to correspond to a peptides binding motif were not tested in the relevant assembly assay but tested only for CTL recognition. Cells from 82 adult Gambians and 53 Gambian children, all exposed to malaria, were used in the course of these studies. The children and adults were HLA typed using cellular or molecular techniques as described previously (Hill et al., 1992). Peptides were incubated with cells either singly, or in pools as described (Hill et al., 1992) at concentrations of 10-100μM. Peptides could either be left with the cells for the duration of restimulation (as reported in Hill et al., 1992) or washed off after an hour. Cells were cultured for 1-3 weeks before a standard CTL chromium release assay (Hill et al., 1992) was performed using HLA matched or autologous B cell line targets pre-pulsed with the peptide to be tested. Peptides were tested either singly or in pools for CTL recognition. 10% or greater was regarded as a significant level of specific lysis.

Page 6, line 20, please rewrite the paragraph as follows:

ii) HLA-B7. CTL from one child with HLA-B7 showed significant lysis of HLA-B7 matched target cells pre-pulsed with the peptide pool cp6, cp6.1 and cp6.2. These peptides are encoded by allelic variants of the same region of the circumsporozoite protein gene (Doolan et al., 1992) identifying this sequence as an HLA-B7 restricted epitope. All three peptides bound to HLA-B7 in the HLA assembly assay. CTL from one adult (Z174) also recognised a pool of four peptides containing cp6, cp6.1, cp6.2 and cp21.

Page 8, line 29 to page 9, line 2, please rewrite the paragraph as follows:

This work identifies TRAP as a *P. falciparum* antigen which induces cytotoxic T lymphocyte responses in individuals exposed to endemic malaria. TRAP is expressed on sporozoites (Cowan et al.) as well as blood-stage malaria parasite and will therefore be present in the infected liver cell. We show here that TRAP contains CTL epitopes for three very common

HLA class I antigens (HLA-A2, -B8 and -B17) and therefore the induction of CTL to TRAP may be an important requirement of an effective CTL-inducing vaccine against *P. falciparum*.

Page 9, line 3, please rewrite the paragraph as follows:

As there is evidence that better CTL responses may be induced *in vivo* using epitopes or epitopes with a limited amount of flanking sequence than by using the whole antigen, the epitopes used here may be particularly valuable for the induction of CTL responses *in vivo* (Lawson et al., 1994).

Page 9, line 9, please rewrite the paragraph as follows:

This specification identifies for the first time the existence of CTL responses to the antigen TRAP in humans exposed to *P. falciparum* parasites. It is known from studies of rodent malaria and indirectly from studies of human *P. falciparum* malaria (Calvani et al., 1994) that CTL are likely to play a protective role but the target antigens of these CTL have been unclear. By identifying TRAP as a target of CTL responses in humans we identify it as a favourable antigen for inclusion in a vaccine designed to induce protective CTL responses. Moreover, we show here that TRAP contains conserved CTL epitopes for the very common class I antigen, HLA-A2, which is the most prevalent HLA-A or -B molecule in Caucasians, making TRAP of particular importance for immunization through CTL of Caucasian populations.

Page 9, line 24 to page 10, line 12, please rewrite the paragraph as follows:

There are several means by which the CTL epitopes identified here may be used to stimulate an immune response *in vivo* in humans. Either the peptides, or longer peptides containing them, can be used alone or with an adjuvant, such as incomplete Freund's adjuvant (Kast et al., 1991) or QS-21 (Newman et al., 1992) or NAGO (Zheng et al., 1992) or AF (Raychaudhuri et al., 1992), or as peptides with a lipid-tail added (Deres et al., 1989), a means that has been shown to enhance CTL induction *in vivo*. Alternatively, the epitopes can be delivered by recombining nucleotides encoding them into a gene coding for a particle such as a

recombinant Ty-virus-like-particle (Layton et al., 1993) or a recombinant hepatitis B virus antigen particle (Tindle et al., 1994). Alternatively, nucleotides encoding these epitopes can be incorporated into a recombinant virus such as a vaccinia virus or an attenuated vaccinia virus (Cox et al., 1993). Another means is to generate a recombinant bacterium such as a recombinant *Salmonella* containing nucleotides encoding these epitopes (Chatfield et al., 1992). Another means is to incorporate nucleotides encoding the epitopes identified into an expression vector, such as a DNA vaccine (Ulmer et al., 1993), that can express these epitopes after immunization. Finally, ribonucleotides coding for these epitopes can be used as an RNA-based vaccine (Martinon et al., 1993) to express these epitopes *in vivo*.

Page 10, line 16, please rewrite the paragraph as follows:

Although secondary (or recall) CTL responses to a variety of infectious micro-organisms can now be detected (e.g. as described for malaria above), CTL cannot be grown in this way from individuals unexposed to antigen or microorganism (Hill et al., 1992 and unpublished data). We describe here a novel method of growing "primary" CTL i.e. from previously unprimed individuals. This method can be employed for generating cell lines and clones which may be useful in various ways: to identify potential epitopes amongst a pool of peptides which bind to an HLA class I molecule; to identify peptides presented by HLA molecules on the surface of a cell using a CTL assay; for *in vivo* therapeutic use for the treatment of infectious or neoplastic disease. We demonstrate this method by describing the generation of CTL lines and clones to two peptides from *P. falciparum* from the lymphocytes of three individuals who have not been exposed to or infected by this parasite.

Page 10, line 35 to page 11, line 9, please rewrite the paragraph as follows:

The method described here for inducing primary CTL responses *in vitro* may be particularly useful in cancer immunotherapy. Studies in mice have demonstrated the potential of therapy with *ex vivo* cultured CTL (Greenberg et al., 1991), and human tumor-specific CTL have been identified in the peripheral blood or tumor-infiltrating lymphocytes from patients with

melanoma and renal cell carcinoma (Cerottini et al., 1992 and Koo et al., 1993). Additionally, induction of CTL against viral antigen epitopes *in vitro* may be useful in the therapy of viral infections such as HIV (Riddell et al., 1994).

Page 11, line 12, please rewrite the paragraph as follows:

Peripheral blood mononuclear cells from individuals never exposed to malaria were separated on Ficoll-hypaque and prepulsed for 2 hours with 20-100μM peptide. In the case of two HLA-A2 individuals the peptide was cp36; in the case of one HLA-B8 individual the peptide was tr43. The cells were then washed once and 5 million cells were incubated in a 2 ml well (in a standard humidified incubator with 5% CO_2) in α-MEM (minimal essential medium, GIBCO, UK) with autologous heat-inactivated human serum and 2μg per ml of keyhole limpet haemocyanin (KLH, Calbiochem, California, USA). The addition of the latter was based on our previous showing that this preferentially stimulates the $CD45RA^+$ (native) subset of CD4 T lymphocytes (Plebanski et al., 1992 and Plebanski et al., 1994). This CD4 T lymphocyte subset has been shown previously to promote CD8 T cell activity (Morimoto et al., 1986).

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Page 13, please replace in its entirety with the following new page 13:

TABLE

label	SEQ ID No.	Sequence										Position
HLA-A2		1	2	3	4	5	6	7	8	9	10	
tr26 *	1	H	L	G	N	V	K	Y	L	V		3
tr29	2	L	L	M	D	C	S	G	S	I		51
tr39 *	3	G	I	A	G	G	L	A	L	L		500
ls10	4	I	L	Y	I	S	F	Y	F	I		4
ls11	5	Y	I	S	F	Y	F	I	L	V		6
ls19	6	G	I	Y	K	E	L	E	D	L		1801
ls23	7	H	I	F	D	G	D	N	E	I		1883
cp36 *	8	Y	L	K	T	I	Q	N	S	L		334
cp37 *	9	Y	L	Q	K	I	Q	N	S	L		334
cp38 *	10	Y	L	Q	K	I	K	N	S	L		334
cp39 *	11	Y	L	N	K	I	Q	N	S	L		334
HLA-B8												
cp43	12	L	R	K	P	K	H	K	K	L		134
cp44	13	L	K	K	I	K	N	S	I	S		335
cp45	14	Q	V	R	I	K	P	G	S	A		358
cp46	15	A	N	K	P	K	D	G	L	D		366
tr42 *	16	A	S	K	N	K	E	K	A	L		107
tr43 *	17	K	N	K	E	K	A	L	I	I		109
HLA-B7												
cp6 *	18	M	P	N	D	P	N	R	N	V		300
cp6.1 *	19	M	P	N	Y	P	N	R	N	V		300
cp6.2 *	20	M	P	N	N	P	N	R	N	V		300
ls6	21	K	P	I	V	Q	Y	D	N	F		1786
sh1	22	I	P	S	L	A	L	M	L	I		7
sh6	23	M	P	L	E	T	Q	L	A	I		77
cp21	24	N	P	D	P	N	A	N	P	N	V	120
tr6	25	N	P	E	N	P	P	N	P	D	I	348
tr13	26	I	P	D	S	I	Q	D	S	L		164
tr15	27	E	P	A	P	F	D	E	T	L		529
tr21	28	G	P	F	M	K	A	V	C	V		228

Page 14, please replace in its entirety the following new page 14:

label	SEQ ID No.	Sequence 1	2	3	4	5	6	7	8	9	10	Position
HLA-B17												
cp48	29	L	S	V	S	S	F	L	F	V		8
cp55	30	G	S	A	N	K	P	K	D	E	L	364
cp56	31	C	S	S	V	F	N	V	V			388
ls36	32	N	S	E	K	D	E	I	I			28
ls37	33	G	S	S	N	S	R	N	R	I		42
ls39	34	V	S	Q	T	N	F	K	S	L		92
ls40	35	K	S	L	L	R	N	L	G	V		98
ls42	36	Q	S	D	S	E	Q	E	R	L		179
ls45	37	R	T	K	A	S	K	E	T	L		1187
ls48	38	H	T	L	E	T	V	N	I			1742
ls49	39	I	S	D	V	N	D	F	Q	I		1749
ls50	40	I	S	K	Y	E	D	E	I			1757
ls51	41	I	S	A	E	Y	D	D	S	L		1764
ls53	42	K	S	L	Y	D	E	H	I			1854
ls54	43	L	S	E	D	I	T	K	Y	F		1898
ls55	44	T	K	Y	F	M	K	L				1902
tr57	45	K	T	A	S	C	G	V	W	D	EW	240
tr58	46	G	T	R	S	R	K	R	E	I	L	260
tr59	47	S	S	V	Q	K	P	E	E	N	I	311
tr60	48	D	S	E	K	E	V	P	S	D	V	367
tr61	49	Y	S	P	L	P	P	K	V	L		415
tr62	50	E	S	D	N	K	Y	K	I	A		490
tr63	51	A	T	P	Y	A	G	E	P	A		523
tr64	52	E	T	L	G	E	E	D	K	D	L	535

* Peptide identified as an epitope for a secondary cytotoxic T lymphocyte response.